ACTA
PROTOZOOLOGICA

Acta Protozool. (2014) 53: 139–144
http://www.eko.uj.edu.pl/ap
doi:10.4467/16890027AP.14.013.1594

Review paper

Taste and Smell in *Acanthamoeba* Feeding

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Abstract. The ability to detect food in the environment is an essential function among all living organisms and must have arisen at the beginning of life. The anatomical, morphological and molecular features involved in taste and smell may differ among disparate groups, but the principle of chemoreception is conserved. Here we debate the sense of taste and smell in the feeding behaviour of the unicellular protist, *Acanthamoeba*.

Key words: *Acanthamoeba*, chemoreception, protist, model organism.

ACANTHAMOEBA: A MODEL ORGANISM

Acanthamoeba was discovered as a culture contaminant of the fungus, *Cryptococcus pararoseus* in 1930 by Castellani (Castellani 1930). In recent decades, *Acanthamoeba* has gained significant attention by the scientific community. It is used as a model organism to study the molecular basis of motility, ability to capture prey by phagocytosis, and host-pathogen interactions. It is also described as the Trojan horse of the microbial world, an organism involved in hyperparasitism (parasite within a parasite), as well as a component of the ecosystem (reviewed in Khan 2009). Recently, it has been suggested to be a 'genetic mixer', i.e., an environmental sanctuary for bacterial/viral pathogens facilitating genetic exchanges which affect their virulence, as well as cellular differentiation. Moreover, the discovery of Mimivirus (closer to prokaryotes than viruses) inside *Acanthamoeba* challenged scientific theories on evolution among virologists and evolutionists (Raoult *et al.* 2004, Yamada 2011, Colson *et al.* 2012). The purpose of this article is to discuss the potential of *Acanthamoeba* as a model organism of yet another physiologically important function: that is sensing food. This property is highlighted in the context of selective feeding behaviour of this important unicellular organism.

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SENSING TASTE AND SMELL

Taste and smell are important sensory mechanisms designed to sense food from a distance and finally examine it before allowing its entry into the body of the cell or an organism. Both invertebrate and vertebrate animals are able to taste and smell. For example, mam-

mals use specialized gustatory cells on the tongue to taste and cells in the nasal cavity to detect volatile chemicals during smelling. The senses of taste (gustation) and smell (olfaction) are both chemical senses to respond to specific chemicals in the environment. Our perception is that a useful diet both smells and tastes good, while harmful substances smell and taste bad. As both taste and smell are chemical senses, they are detected by receptors (chemoreceptors) that provide chemical composition of substances. Given the vital nature of these senses for any living organism, it is likely they are the first senses that develop in life (Wicher 2012). Hence, the anatomical, morphological and molecular features involved in taste and smell may differ amongst disparate groups, but the principle of this fundamental sense, i.e., chemical sensory systems have most likely been preserved throughout the evolution of life on earth (Biswas *et al.* 2009, Gees *et al.* 2010, Nordström *et al.* 2011). Moreover, the functional separation between these two systems is blurred in some cases and there are more subsystems serving chemosensory roles. From an evolutionary perspective, taste and smell were only separated in land animals. For sea creatures, there is only one chemical sense because all chemicals get dissolved in the same medium, i.e., water, and there is no need for two separate senses. For example, catfish have chemoreceptors all along their body to detect taste. On the other hand, flies have receptors on their feet and detect food particles by landing on them.

Among the single-celled eukaryotes, Amoebae evolved early in life (Philippe *et al.* 2000). In this diverse group of organisms, *Acanthamoeba* [amoeba with spines (Greek *acanth* means spine) on its surface] is a free-living amoeba, commonly found in soil and freshwater environments, but it is also an opportunistic protist pathogen and is known to cause granulomatous amoebic encephalitis and keratitis. Although the single-celled microorganisms are estimated to have emerged on the earth approximately 3–4 billion years ago, based on ribosomal RNA sequences, it is estimated that *Acanthamoeba* diverged from the main line of eukaryotic descent, sometime between the divergence of yeast ($\sim 1.2 \times 10^9$ years ago) and the divergence of plants and animals ($\sim 1 \times 10^9$ years ago) (Roger and Hug 2006, Khan 2009). Being a free-living amoeba, *Acanthamoeba* feeds on soil bacteria on land, as well as freshwater bacteria (Preston *et al.* 2001), thus it can serve as a model organism to study chemosensory systems. As distinction between taste and smell is not necessary for aquatic metazoans, it is likely that this is also the case for aquatic protists such as amoebae. Regardless of however the environment, i.e., solid substratum such as agar or surface microlayer of natural ponds, feeding occurs by phagocytosis, an apparent characteristic of most amoebae (Preston *et al.* 2001).

Like other microbes, *Acanthamoeba* exhibits chemosensory responses and possesses receptor(s) in its plasma membranes to detect chemo-attractants (Schuster and Levandowsky 1996). For example, *A. castellanii* responds to a variety of bacterial products such as lipopolysaccharide, lipid A, and cyclic AMP, lipoteichoic acid, *N*-acetyl glucosamine, and chemotactic peptide formyl-methionyl-leucyl-phenylalanine by moving actively toward the attractant. Mannose or mannosylated bovine serum albumin yields non-significant responses. Treatment of *A. castellanii* with trypsin reduces the directional response of cells, suggesting the amoeba plasma membrane has receptors, sensitive to these chemo-attractants, which are different from the mannose-binding protein of *Acanthamoeba*. The mannose-binding protein is involved in binding bacteria to the plasma membrane of *Acanthamoeba* during phagocytosis (Schuster and Levandowsky 1996), as well as the amoeba binding to the host cells during infection (Alsam *et al.* 2003, Garate *et al.* 2004).

PREFERENTIAL FEEDING BEHAVIOUR OF *ACANTHAMOEBA* IS NOT UNUSUAL AND COMMONLY OBSERVED IN OTHER SPECIES

For many researchers, preferential feeding behaviour of *Acanthamoeba* is mystifying. While *Acanthamoeba* is known to not only feed on bacteria (Weekers *et al.* 1993), but yeast (Allen and Dawidowicz 1990), archea (Iqbal *et al*. 2012) and other protists are also grazed upon (Khan 2009). In addition to food particles, *Acanthamoeba* avidly takes up non-nutritive particles such as polystyrene latex spherules of microscopic size (Korn and Weisman 1967, Chambers and Thompson 1976, Yan *et al*. 2004), however it possesses the ability to discriminate internally between digestible and indigestible particles (Bowers 1983).

Given a choice, *Acanthamoeba* preferentially feeds on Gram negative bacteria (Weekers *et al.* 1993, Rosenberg *et al.* 2009), indicating the presence of external chemosensory discrimination, as well as internal discrimination. Moreover, *Acanthamoeba*-bacteria are in-

volved in complex interactions, the outcome of which is dependent on the virulence properties of *Acanthamoeba*, the virulence properties of bacteria and the environmental conditions (Greub and Raoult 2004, Khan 2006). For the scope of this article, here we focus on the role of *Acanthamoeba* as a bacterial predator only, while the role of amoeba as a vector/biological reservoir of bacterial pathogens has been covered elsewhere and is not discussed (Greub and Raoult 2004; Khan 2006, 2009). In its role as a bacterial grazer, it is assumed that processes involved in bacterial uptake are mediated by *Acanthamoeba*, fulfilling amoebal nutritional requirements, whereas bacteria play a rather passive role and fall prey to this microbial predator. It is puzzling that *Acanthamoeba* feeds preferentially on Gram negative bacteria but not on Gram positive bacteria (Weekers *et al.* 1993, Rosenberg *et al.* 2009). Even among the Gram negative bacteria, *Acanthamoeba* exhibits selectivity for certain species (e.g., *Escherichia coli* and *Enterobacter aerogenes*), however the basis of this selectivity is not known. It can be postulated that the taste and smell stimuli (pheromones/chemicals) of bacteria and/or presence of specific chemical sensory systems may explain *Acanthamoeba*'s ability to exhibit prey discernment during its feeding behavior. This is not a novel concept as preferential feeding behaviour is common in animals. For example, aversion of raw tomatoes by many of us has been attributed possibly to some of the 400 volatite compounds giving it a harsh smell for some, and/or presence of geranial, 2-methylbutanol, 3-methyl-1-butanol or furaneol contributing to its sweetness (Tieman *et al.* 2012). Similar studies on cilantro have indicated that in addition to smell and flavour, the genetic makeup of an individual play a role in the liking/disliking of cilantro and this is accredited to absence, presence or mutation of receptor genes (Mauer and El-Soheny 2012). For example, people disliking cilantro detect a soapy odor but have a mutated or absent receptor gene which detects the pleasant smelling compound. About 25% of the population cannot taste propylthiouracil, a chemical similar to bitter compounds found in coffee, cabbage, dark beers as they possess the wrong variant of its receptor, TAS2R38 on the tongue (Hayes *et al.* 2011, Drayna 2005). However, its never about a single gene but an array of chemicals or their combinations that are detected by chemical sensory systems that determines preferential feeding behaviour.

Chemical sensory systems are functional complexes of receptors (chemoreceptors) and downstream signaling molecules. Chemoreceptors are transmembrane proteins that transduce a chemical signal in the external environment into an action potential and relay this information intracellularly. Following the binding of molecules to their external surface, chemoreceptors are activated, and transduce an external signal into an intracellular signal. A sensory system to detect chemical senses is present in all living organisms, from simple prokaryotes to highly complex vertebrates. For example, in *Escherichia coli*, chemoreceptors are transmembrane methyl-accepting chemotaxis proteins (Hazelbauer *et al.* 2008) and they sense amino acids using glutamate receptors, while yeast chemoreceptors are G-protein coupled receptors to detect sugar/pheromones (Versele *et al.* 2001), and transient receptor potential channels for sensing aromatic compounds (Nilius and Owsianik 2011). Nematode chemoreceptors are receptor guanylyl cyclases, many G-protein coupled receptors, transient receptor potential channels, and receptors related to glutamate receptors (Croset *et al.* 2010). In vertebrates, these include receptor tyrosine kinases, guanylyl cyclases, many G-protein coupled receptors, transient receptor potential channels, and receptors related to glutamate receptors (Petersen *et al.* 2011, Fulle *et al.* 1995, Sun *et al.* 2009, Kaupp 2010). For multicellular organisms, chemoreceptors can be divided into two major types: (i) ionotropic receptors that are ion channels and are activated immediately by ligand binding, and (ii) metabotropic receptors that activate an intracellular signaling cascade leading to enzyme activation, second messenger production, activation of ion channels etc. (Biswas *et al.* 2009, Gees *et al.* 2010, Nordström *et al.* 2011). Among ionotropic receptors, P2X receptors are activated by adenosine triphosphate (ATP), leading to opening of a non-selective cation pore, membrane depolarization and calcium influx (North 2002). ATP plays an important physiological role as a transmitter molecule in processes including sensation of pain, taste, breathing and inflammation via activation of P2X receptors. The identification of P2X receptors in *Distyostelium* suggests its early lineage (Ludlow *et al.* 2008). In addition to possible presence of P2X receptors, *Acanthamoeba* are also known to exhibit ecto-ATPases (ATP degradating enzymes), i.e., glycoproteins present in their plasma membranes and their active sites face the external medium rather than the cytoplasm and hydrolyse extracellular ATP, regulating extracellular ATP-dependent processes and protection from cytotoxic effects of ATP (Sissons *et al.* 2004). Additionally, phagocytosis in *A. castellanii* is associ-

ated with elevated levels of adenosine monophosphate (AMP) (Edwards and Doulah 1982), much of which is found extracellularly (Edwards and Lloyd 1977).

The directional movement of organisms in response to external stimuli is well known (Grishanin and Bibikov 1997, Armitage 1999, Taylor *et al.* 1999, Vickers 2000, Schweinitzer and Josenhans 2010) and this has been described in the context of aerotaxis (stimulation by oxygen), anemotaxis (wind), barotaxis (pressure), chemotaxis (chemicals), galvanotaxis (electrical current), gravitaxis (gravity), hydrotaxis (moisture), magnetotaxis (magnetic field), osmotaxis (osmolarity), phototaxis (light), rheotaxis (fluid flow), thermotaxis (temperature changes), and thigmotaxis (physical contact) to name a few. The complexity doesn't end there. Even the same type of chemoreceptor exhibits variability in receptor topology and the type of sensor domain. For example, bacterial methylaccepting chemotaxis proteins exhibit six major topologies and differ being membrane-bound or cytoplasmic and vary in the location of ligand binding (Krell *et al.* 2011). The overall process of any form of taxis involves, the input signal (such as cGMP), the receptor (such as G-protein-coupled receptors), and mode of activation, the presence of additional cytoplasmic signal transduction proteins or the motor mechanism (such as small GTPases and adelylyl cyclase activation, affecting distribution of myosin in the cell localizing the construction of the actin cytoskeleton), leading to a desired effect (e.g., actin polymerization and directed cell movement) for the organism. The absence of any single molecule in this pathway would result in 'no taxis'. But how it affects the preferential feeding behaviour of *Acanthamoeba*, is not known. *Acanthamoeba* exhibits preferential feeding behaviour only in the presence of a variety of available foods. In the absence of any choice, it may attack any available bacteria, be that Gram negative *E. coli* (Alsam *et al.* 2006), Gram positive methicillin *Staphylococcus aureus* (MRSA) (Huws *et al*. 2006), spore-forming bacteria such as *Bacillus anthracis* (Dey *et al.* 2012), and thermophilic *Sulfolobus* spp. (Iqbal *et al.* 2013). However, cannibalism is prevented by the secretion of proteins (Khan 2009). The question is, if there is a choice of food, what determines *Acanthamoeba*'s liking? Even if we focus on chemotaxis as a feature to locate food and ignore other types of taxis, the molecular and cellular mechanisms underlying odor and pheromone sensing in *Acanthamoeba* are not known. For instance, bacteria produce various types of pheromones (Lacey and Dutkiewicz 1994, Chanda 1996, Chang *et al.* 2010) and possibly hundreds of terpenes (Cane and Ikeda 2012), which are regulated by environmental and/or physiologically-relevant conditions. To this end, *Acanthamoeba*'s preferential feeding behaviour may be determined by: (i) types of pheromones/terpenes produced by certain bacterial species that are preferred by *Acanthamoeba*, (ii) absence of desired pheromones/terpenes and/or intrinsic presence of deterrent pheromones/terpenes in disfavored bacteria, (iii) inducible expression of averted pheromones/terpenes in certain bacterial species as opposed to others. In addition, chemoreception of *Acanthamoeba* can be highly diverse and requires detailed understanding of the signal, receptor, intracellular signal transduction cascade and motor mechanisms leading to a functional response.

Overall, the likely explanations of *Acanthamoeba*'s preferential feeding of Gram negative bacteria such as *E. coli* and *E. aerogenes* are as follows: (i) the diversity of odor/pheromones/terpenes of bacteria or by-product of bacterial metabolism may appeal/repel *Acanthamoeba* to certain bacterial species compared with other microbes, (ii) the presence and/or absence of specific functional chemoreceptors on the extracellular surface of *Acanthamoeba* to locate food, (iii) size and morphological characteristics of the prey, (iv) biochemical properties of the bacterial surface elements such as capsule, lipopolysaccharide, outer membrane proteins, peptidoglycans (Alsam *et al.* 2006, Humann and Lenz 2009), may stimulate amoebae taste buds, or depending on the structural and biochemical properties inhibit detection and uptake, and (v) the presence and/or absence of lytic enzymes endogenous to *Acanthamoeba* (such as glycosidases, amidases, endopeptidases, exopeptidases, DNAses, RNAses, lipases) required to degrade a particular prey or its molecular structures may determine *Acanthamoeba* preferential feeding habits. Another important area of study is what attracts *Acanthamoeba* to human cells as food source and whether such senses determine its species specificity, tissue specificity, and cellular specificity and how such a sense is linked to the underlying molecular mechanisms to initiate degradative processes resulting in host damage contributing to its pathogenicity. Whether certain amoebal species/genotypes lack sensory system receptor genes or possess variants and its link to a particular host with an unwanted damage is a speculative but interesting topic for further research. Another important area of study is the ability of *Acanthamoeba* to avidly takes up non-nutritive particles such as polystyrene latex spherules, albeit subjected to internal discrimination (Korn and

Weisman 1967, Chambers and Thompson 1976, Yan *et al.* 2004). Clearly, there is some peculiar bypass of the normal chemosensory mechanism for selective attachment and uptake of particulates when objects such as polystyrene latex spherules are so avidly consumed and this should be explored in future studies.

CONCLUSIONS

In summary, certain bacterial chemo-attractants may prove to be the 'smell of love' to chemo-attract *Acanthamoeba*. This together with the presence of specific receptors ("sensory organs for taste and smell") to detect favourable prey and subsequent motor mechanisms may determine their preferential feeding behaviour of certain bacterial species. Being a relatively simple organism, *Acanthamoeba* offers a unique opportunity to understand the physiological roles of this critically important property. Future studies will determine precisely the molecular information of bacterial scent and amoebal chemoreceptors (G-protein-coupled receptors), which will help us understand the biology of this fascinating organism occupying an important position in the evolution of eukaryotic organisms, and the findings will help elucidate the biological benefits of this important function in vertebrates.

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Received on 21st June, 2013; revised on 9th September, 2013; accepted on 14th September, 2013